Article

Sexual dimorphism and length weight relationship in chestnut crab, *Cardisoma carnifex* Herbst, 1796 (Decapoda: Gecarcinidae) of Parangipettai stock, Tamilnadu, India

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Received 21 December 2024; *Accepted 31 January 2025*; *Published online 11 February 2025*; *Published 1 September 2025*

Abstract

The present study was conducted in the Parangipettai mangrove area of Tamilnadu, India, from November 2021 to April 2022, which aims to investigate the relationships between carapace length-weight and carapace width-weight of the chestnut crab, *Cardisoma carnifex*. A total of 129 crabs were examined, including 66 females and 63 males. Results indicated that males had a mean body weight of 343 ± 81.91 g, while females weighed 377.86 ± 86.28 g on average. Furthermore, females exhibited a significantly larger mean carapace length (6.32 ± 2.35 cm) compared to males (5.85 ± 2.10 cm). The correlation coefficient (r^2) highlighted a stronger association between body weight (BW) and carapace width (CW) than BW and carapace length, indicating that variations in body weight were predominantly influenced by changes in carapace width. Positive relationships between carapace length and carapace width were observed in males (b = 1.0733, r^2 = 0.9467), females (b = 1.0136, r^2 = 0.9658), and both sexes combined (b = 1.0544, r^2 = 0.9532). This sexual dimorphism, with females being larger than males, may suggest an adaptation associated with increased egg production. The study's findings provide valuable baseline data on the morphological characteristics of *C. carnifex* in the Parangipettai stock. Such information is essential for establishing ongoing monitoring and management strategies for this crab species, contributing to the conservation of biodiversity in the region.

Keywords carapace length-weight relationship; allometric growth; *Cardisoma carnifex*; sexual dimorphism.

Arthropods
ISSN 2224-4255
URL: http://www.iaees.org/publications/journals/arthropods/online-version.asp
RSS: http://www.iaees.org/publications/journals/arthropods/rss.xml
E-mail: arthropods@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Terrestrial or land crabs belonging to the family Gecarcinidae, order Decapoda, class Crustacea, are characterized by their square-shaped carapace and primarily inhabit land as adults, occasionally venturing into aquatic environments for breeding and larval development. The family Gecarcinidae encompasses seven genera, with the *Cardisoma* genus comprising four species: *C. armatum*, *C. carnifex*, *C. crassum*, and *C. guanhumi*. Among these species, *C.carnifex*is widely distributed across coastal regions, spanning from Eastern Africa and the Red Sea through the Indo-Pacific to the Line Islands and the Tuamotu Archipelago (Haig, 1984). Its range extends to northern Australia and the Cocos (Keeling) Islands (Davie, 2002). In India, *C. carnifex* has been reported along the coasts of Gujarat (Shukla et al., 2013) Kerala (Dev Roy, 2013) Tamilnadu (Varadharajan et al., 2013), and West Bengal (Alam et al., 2020), Kerala, Tamilnadu, and West Bengal. Despite its extensive distribution, information regarding the length-weight relationship and sexual dimorphism of *C. carnifex*, particularly in the context of the Parangipettai mangroves, has received limited attention or remains largely unexplored.

Understanding the relationship between length and weight is crucial in assessing the natural stocks of finfish and shellfish, as highlighted by Enin (1994) and Stergou and Moutopoulos (2001). This relationship serves various purposes such as converting individual lengths to weights, estimating mean weights for given lengths, converting growth equations, and comparing morphology among populations or species. Body weight, total length, carapace length, and width are among the most commonly used parameters in crustaceans, as noted by Sukumaran and Neelakantan (1997). As crustaceans develop, their body size increases, a phenomenon known as relative growth (Thanamalini and ShylaSuganthi, 2018). Variations in growth patterns between sexes of the same species or among different populations or species hold biological significance (Sullivan, 1998; Fransozo et al., 2009; Brian, 2005).The present study aimed to investigate the relative morphological variations between male and female *C. carnifex* in the Parangipettai mangrove area of Tamilnadu, India. Additionally, the study aimed to explore and discuss the sexual dimorphism observed in this species. This research contributes to our understanding of the morphological differences between genders and sheds light on the ecological and evolutionary factors influencing sexual dimorphism in *C. carnifex* populations.

2 Materials and Methods

2.1 Animal collection and morphological measurements

A total of 129 live *C. carnifex* specimens were collected from the vicinity of the Parangipettai mangrove forest, located at coordinates 11°29'24.11″N 79°45'57.51"E and 11°28′25.34"N 79°46'34.71"E, through handpicking between November 2021 and April 2022. Upon collection, the crabs were temporarily sedated in iced water, followed by rinsing and preservation in 70% alcohol for subsequent identification. Gender determination was conducted by assessing claw and abdomen size, as well as the presence of the appendix masculina within the abdomen. Frequency distribution analysis was performed on the major chela (claw) of the specimens. Various measurements were taken using a digital calliper (Zhart, India) with 0.1mm accuracy, including CL, CW, CFW, ECL, IOD, ATL, ASW, CW, CJW, CDL, RMC, LMC, FMW, SMW, TMW, FoMW, FML, SML, TML, and FoML. Additionally, tissue samples were dissected from three individuals for DNA isolation and preserved in 95% ethanol for further genetic analysis. The detailed information regarding the measurements and specimens is provided in Table 1 and Fig. 1. These procedures were essential for obtaining comprehensive data on the morphological characteristics and genetic profiles of the collected *C. carnifex* specimens from the Parangipettai mangrove area.

Table 1 Corporal measures analyzed in *C. Carnifex*.

Dorsal Region	Ventral Region	Pereiopods
BW- Body weight	ATL - Abdomen total length	CW - First movable chela width
CL - Carapace length	ASW - Abdomen first suture width	CJW - Width of the joining the propodus and the movable chela thigh
CW - Carapace width		CDL - Chela dactylus length
CFW - Carapace final width		RMC/LMC - Right and left major movable chela
ECL - Eye cavity length		FMW - First pereiopod merus width
IOD - Inter orbital distance		SMW- Second pereiopod merus width
MPW- Medial peduncle width		TMW- Third pereiopod merus width
		FoMW- Fourth pereiopod merus width
		FML - First pereiopod merus length
		SML- Second pereiopod merus length
		TML- Third pereiopod merus length
		FoML- Fourth pereiopod merus length

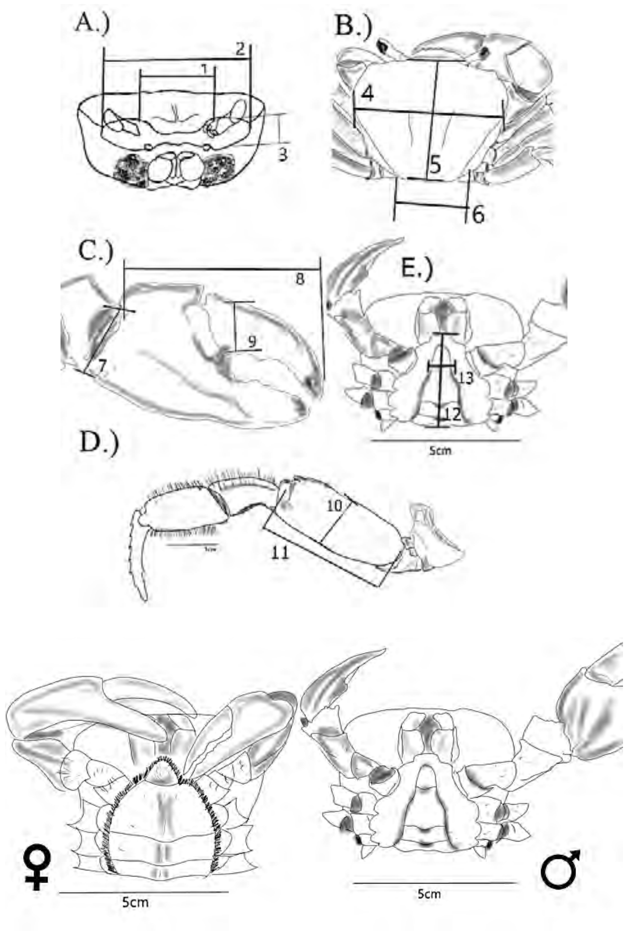

Fig. 1 Corporal measures analyzed in *C. carnifex*. (A-1)- ECL; (A-2)-IOD; (A-3)-MPW; (B-4)-CW; (B-5)-CL; (B-6)-CFW; (C-7)-CJW; (C-8)-CDL; (C-9)-CW; (D-10)-MW; (D-11)-ML; (E-12)-ATL; (E-13)-ASW.

2.2 Data analysis

To determine allometric growth, logarithmic transformation was applied, expressed as:

$Y=\log(a) + b \log(X)$

Here, '*a*' and '*b*' were estimated through linear regression, where:

'*a*' represents the intercept of the regression curve.

'*b*' represents the regression coefficient (slope).

The 'b' value indicates the relative growth constant:

$b=1$ signifies isometric growth.

$b>1$ indicates positive allometric growth.

$b<1$ suggests negative allometric growth (Hartnoll, 1982; Tessier, 1960).

To assess factorial variation between male and female individuals, a one-way ANOVA (Analysis of Variance; Zhang and Qi, 2024) was conducted (Zar, 1996). This statistical analysis is used to determine if there are any statistically significant differences between the means of two or more independent groups. These analytical techniques were employed to understand the growth patterns and variations between male and female *C. carnifex* individuals, providing valuable insights into their morphological development and sexual dimorphism.

2.3 Molecular identification

Total genomic DNA was extracted from tentacle muscle tissue using the method outlined by Sambrook et al. (2001). Following extraction, the DNA concentration was determined using a UV spectrophotometer. The cytochrome c oxidase subunit I (COI) gene was targeted for amplification in a 50 µl reaction volume. The PCR mixture included 100 ng of template DNA, 10 µmol of each specific primer, 200 µM of each dNTP, 1.0 units of Taq DNA polymerase, and 1× Taq buffer containing 1.5 mM $MgCl_2$. Universal primers *LCOI1490F1* (5'GGTCAACAAATCATAAAGATATTGG3') and *HCOI2198* (5'TAAACTTCAGGGTGACCAAAAAATCA3') (Folmer et al., 1994) were utilized for COI gene amplification. The PCR conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 54°C for 45 sec, extension at 72°C for 60 sec, and a final extension step at 72°C for 10 min. The PCR products were visualized on 1.5% agarose gel. Subsequently, the amplified DNA fragments were sent to a commercial sequencing facility (Eurofins, Bangalore) for sequencing analysis.

3 Results

3.1 Distinguishing characteristics and sex ratio

Cardisoma carnifex, commonly known as the "chestnut crab," possesses distinctive features that contribute to its unique appearance and characteristics. The body shape of this crab species is often described as heart-shaped, with a large size and maroon coloration (Fig. 2). Notably, there is sexual dimorphism in the size and shape of the claws, with males typically exhibiting larger and more robust claws compared to females. The dorsal surface of the carapace is characterized by a blackish-grey coloration, while the ventral surface tends to be yellowish-white. Additionally, the coxa, which is a segment of the crab's limb, displays a yellowish-white color on both the dorsal and ventral sides. In its juvenile stage, *C. carnifex* is known for its vibrant and striking coloration, including a purple-blue carapace and orange-red legs. This colorful appearance has contributed to its popularity in the pet trade. However, as the crab matures, these vivid colors tend to fade, and adult females may exhibit duller hues compared to males. Male and female cannot be easily distinguished by observing in their burrows.

Fig. 2 *Cardisomacarnifex* female.

3.2 Molecular identification

The DNA sequence of the voucher specimen TRL641 was submitted to the NCBI via the BankIt protocol and was assigned the accession number ON197889. The sequence obtained from this specimen was 487 base pairs (bp) in length, with partial coverage at both the 5′ and 3′ ends. Importantly, there were no insertions, deletions, or premature stop codons observed in this sequence. Upon analysis using NCBI-BLAST, the sequence showed a high degree of similarity, with a 98.77% match, and achieved 100% sequence coverage when compared to known sequences in the NCBI database. This result conclusively identified the specimen as belonging to *C. carnifex* without any ambiguity.

3.3 Morphometric variation analysis

Analysis of morphological characteristics revealed significant differences between males and females. Specifically, the mean carapace length (CL) of males (5.85 ± 2.10 cm) was significantly smaller than that of females (6.32 ± 2.35 cm). Similarly, the mean carapace width (CW) of males (7.83 ± 2.36 cm) was notably smaller compared to females (8.46 ± 2.47 cm). Additionally, the mean body weight (BW) of males (343 ± 81.91 g) was lower than that of females (377.86 ± 86.28 g). Among the 13 morphometric characters analyzed, several parameters exhibited significant variation between males and females. These included %CL(CW), %ASW(ATL), %CFW(CW), %CFW(CL), %FMW(FML), %MaCW(MaCDL), %MaCJ W(MaCDL) and %FoMW(FoML). However, no significant variation between sexes was observed in %ECL(IOD), %SMW(SML), %TMW(TML), %MiCW (MiCDL) and %MiCJW(MiCDL) (Table 2).

Regression analysis of body weight (BW) against carapace width (CW) revealed a significantly stronger linear relationship in females compared to males in the present study. However, the coefficient of determination (r^2) indicated a weaker relationship between body weight and carapace length (CL), with significant differences observed between males and females. The results of the BW-CL (body weight-carapace length) and BW-CW (body weight-carapace width) relationships derived from regression analysis are summarized in Table 3. The correlation coefficient (r^2) values indicate the strength of the relationship between body weight and carapace length or width for female, male, and pooled sexes. For the BW-CL relationship, the correlation coefficient (r^2) for the female sample was 0.6135, followed by 0.3729 for males and 0.5579 for the pooled sexes. This suggests a moderate correlation between body weight and carapace length in females, while the correlation is weaker in males and the overall population. In contrast, for the BW-CW relationship, the correlation coefficient (r^2) for the female sample was 0.6406, followed by 0.4892 for males and 0.6100 for the pooled sexes. Here, the correlation between body weight and carapace width is stronger across all groups compared to the BW-CL relationship. This indicates that changes in body weight are primarily influenced by variations in carapace width rather than carapace length. Furthermore, the kinship between carapace length (CL) and carapace width (CW) was examined through regression analysis. The results indicate a positive relationship in males (b = 1.0733, r^2 = 0.9467), females (b = 1.0136, r^2 = 0.9658), and the pooled sexes (b = 1.0544, r^2 = 0.9532) (Table 3).

Table 2 Basic morphometric characteristics of *C. Carnifex*((Values in the row sharing common superscript is not significantly different at *P*<0.05 level).

Character	Mean ± SD	
	Male (n=63)	Female (n=66)
BW (g)	343 ± 81.91^a	377.86 ± 86.28^b
%ECL(IOD)	43.49 ± 4.39^a	45.38 ± 4.80^a
%CL(CW)	81.74 ± 6.61^a	83.46 ± 8.30^b
%CFW(CW)	51.36 ± 5.50^a	54.25 ± 5.16^b
%CFW(CL)	42.02 ± 4.11^a	45.01 ± 4.57^b
%ASW(ATL)	27.85 ± 2.25^a	40.04 ± 3.90^b
%FMW(FML)	44.70 ± 4.85^a	48.02 ± 5.60^b
%SMW(SML)	44.49 ± 4.00^a	45.38 ± 4.51^a
%TMW(TML)	43.81 ± 3.80^a	44.81 ± 4.32^a
%FoMW (FoML)	43.57 ± 2.06^a	49.83 ± 2.29^b
%Ma CW(Ma CDL)	29.20 ± 2.21^a	34.09 ± 3.12^b
%Ma CJW(Ma CDL)	33.13 ± 2.58^a	39.21 ± 3.11^b
%Mi CW(Mi CDL)	28.32 ± 1.48^a	30.82 ± 2.25^a
%Mi CJW(Mi CDL)	38.15 ± 3.01^a	40.18 ± 3.54^a

Table 3 Formula for linear regressions and correlation coefficient (r^2) of *C. Carnifex*.

Relationship	Sex	N	Linearized power function	r^2
CW- IOD	M	63	LogCW= 0.9329 + 0.2145 LogIOD	0.9368
	F	66	LogCW= 0.8845 + 0.3148 LogIOD	0.8818
	P	129	LogCW= 0.9035 +0.2754LogIOD	0.9015
CL-CW	M	63	LogCL=-0.2326+1.0733LogCW	0.9467
	F	66	LogCL=-0.1148+1.0136LogCW	0.9658
	P	129	LogCL=-0.1940+1.0544LogCW	0.9532
CFW-CW	M	63	LogCFW=-0.6515+1.1429LogCW	0.9457
	F	66	LogCFW=-0.4127+1.0295LogCW	0.8718
	P	129	LogCFW=-0.5057+1.0733LogCW	0.8981
CFW-CL	M	63	LogCFW=-0.5146+1.1234LogCL	0.9720
	F	66	LogCFW=-0.2345+1.9828LogCL	0.9668

	P	129	LogCFW=-0.3311+1.0302LogCL	0.9650
BW-CL	M	63	LogWt=0.2918+1.9977LogCL	0.3729
	F	66	LogWt=0.3619+1.8781LogCL	0.6135
	P	129	LogWt=0.3473+1.9033LogCL	0.5579
BW-CW	M	63	LogWt=0.3447+1.8715LogCW	0.4892
	F	66	LogWt=0.4080+1.7551LogCW	0.6406
	P	129	LogWt=0.3922+1.7849LogCW	0.6100
CDLMa-CWMa	M	63	LogCDLMa=-0.7929+1.1557LogCWMa	0.7881
	F	66	LogCDLMa=-0.7250+1.1134LogCWMa	0.7147
	P	129	LogCDLMa=-0.7364+1.1209LogCWMa	0.7452
CDLMi-CWMi	M	63	LogCDLMi=-1.0100+1.2594LogCWMi	0.8922
	F	66	LogCDLMi=-0.5267+1.0144LogCWMi	0.8458
	P	129	LogCDLMi=-0.7100+1.1062LogCWMi	0.8575
ATL-ASW	M	63	LogATL=-0.7352+1.1073LogASW	0.9549
	F	66	LogATL=-0.6359+1.1775LogASW	0.7889
	P	129	LogATL=-0.7951+1.1969LogASW	0.8342

3.4 Major chela distribution frequency

The distribution frequency of major chela size asymmetry was examined in the *C. carnifex* population of the Parangipettai stock. In the present study, 57.1% of male crabs exhibited left-handedness in the major chela, while only 36.4% of females displayed left-handedness. The majority of females showed right-handedness, accounting for 53%. Additionally, symmetric chela were observed in 6% of males and 10.6% of females (Fig. 3).

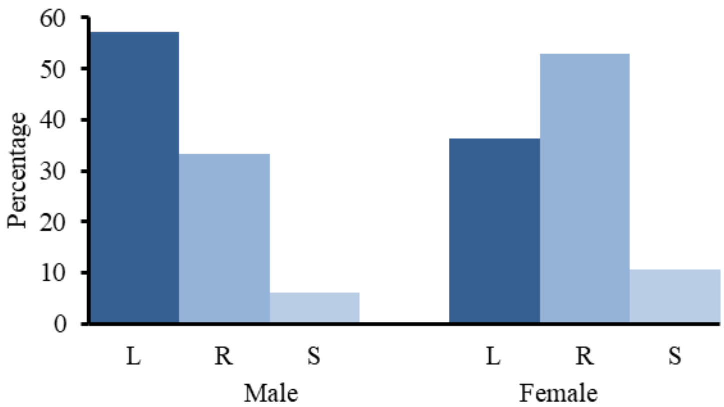

Fig. 3 Distribution of major chela frequency in *C. carnifex* (L- left; R- Right; S- Symmetrical).

4 Discussion

In this study, a total of 129 specimens of *C. carnifex* were examined, consisting of 66 females (51.1%) and 63 males (48.9%). Studies on other crab species, such as *Scylla serrata* and *S. olivacea*, have reported varying sex ratios. For instance, Ali et al. (2004) found an overall sex ratio of 1:0.94 in *S. serrata* from the Khulna region of Bangladesh, while Istiak et al. (2018) observed a sex ratio of 1:0.97 in *S. olivacea* within the Sundarban mangrove forest, also in Bangladesh. The higher proportion of females in *S. olivacea* compared to *S. serrata* and *S. tranquebarica* is attributed to its preference for the intertidal zone, whereas the latter two species exhibit more extensive movement (Ikhwanuddin et al., 2011). In the case of *C. carnifex*, our observations indicate a sex ratio of 0.9:1, with females outnumbering males. However, it's important to note that sex ratios can vary depending on factors such as the size range of the population, seasonal fluctuations (Quinn and Kojis, 1987), and the level of exploitation of the stock (Istiak et al., 2018). These variations underscore the dynamic nature of crab populations and the influence of environmental factors on their demographics.

Females often tend to be larger than males in several species, as reported by researchers such as Castilho et al. (2007), Semensato and Beneditto (2008), and Costa et al. (2010). This female bias in size is often attributed to the behaviour of reproductively active females, who invest more energy into reproduction and egg production, leading to increased body size (Colby and Fonseca, 1984; Conde and Diaz, 1989; Litulo, 2005). In the case of *C. carnifex*, sexual dimorphism in size is evident, with females typically larger than males. This difference in size may be an adaptation to enhance egg production and reproductive success (Castilho et al., 2007). Additionally, sexual differences in cheliped size, common in many crab species, contribute to sexual dimorphism. The extent of sexual dimorphism in cheliped size varies among species and can confer selective advantages in competition for mates, food resources, or territory, as well as in evading predators (Lee, 1995; Razek et al., 2006; Hartnoll, 1982; Koch et al., 2005). Moreover, the shape and size of the female abdomen, serving as the brood chamber for carrying eggs, represent another prominent sexually dimorphic characteristic in crabs (Hartnoll, 1982). Previous studies have identified significant phenotypic variability in shore crabs within relatively small geographical areas (Bentley et al., 2002; Brian, 2005).

This observation is consistent with earlier studies on population variation in *C. guanhumi* (Duarte et al., 2008). Carapace width (CW) is often considered an independent variable in morphometric studies of brachyurans because it undergoes relatively few morphological changes throughout the crab's life (Araújo et al., 2012). This stability in carapace width can be influenced by factors such as latitude, food availability, and habitat characteristics.

This positive correlation suggests that as carapace length increases, carapace width tends to increase proportionally, indicating a consistent growth pattern across genders. The variation in the 'b' values, representing the exponent in length-weight relationships, can be attributed to various factors such as the sizes and length ranges of the samples used, sexual dimorphism, water quality, and food availability (Artigues et al., 2003; Henderson, 2005). The range of 'b' values observed in the current study aligns with values reported in previous research on species such as *Callinectes* and *Penaeus* (Moslen and Miebaka, 2018). In a study of the length-weight relationship of the West African fiddler crab, *Uca tangeri*, Okon and Sikoki (2014) reported a 'b' value of 1.64, which is higher than the findings of the present work. Similarly, Abowei and George (2009) reported 'b' values ranging from 2.35 to 2.54 for *Calinectes amnicola*. Omuvwie and Atobatele (2013) also reported negative allometric growth for *C. amnicola* using carapace width, while positive allometric growth was observed using carapace length, with variations based on sex. In the current study, negative allometric growth was observed in both male and female *C. carnifex* when comparing carapace length to carapace width, carapace final width to carapace width, and carapace final width to carapace length. Positive allometric growth was observed in the abdomen, with adult males exhibiting an isometric relationship (b = 1.1073) and adult females showing positive allometry (b = 1.1775) (Table 3). These findings are consistent with previous studies on other brachyuran crabs (Jasmine, 2012) and highlight the sexual dimorphism present in *C. carnifex*. Moreover, the study demonstrates that other morphometric variables of *C. carnifex* can be used to discriminate between males and females, as shown in Table 2. These variations in size and morphology between male and female crabs reflect adaptations shaped by evolutionary pressures related to reproduction, competition, and survival strategies.

The asymmetry in major chela size suggests a tendency towards a more brachychelous nature in females and a more leptochelous nature in males. The major chela serves multiple purposes for male crabs, acting as a sexual ornament, a weapon, and a feeding structure (Mariappan et al., 2000; Matsuo et al., 2014). However, the dimensions of the cheliped are considered a more reliable indicator of the outcome of struggles and are critically important in defining male mating success (Yasuda et al., 2011). Unlike some other crab species, such as *Carcinus maenas*, which exhibit a preferential occurrence of the major chela on the right side, *C. carnifex* does not show a predisposition to the occurrence of the major chela on a specific body side for either sex (Sneddon and Swaddle, 1999). The relative frequencies of left- and right-handedness of the major chela in male crabs can vary among populations, but generally, right- and left-handed crabs occur about equally (Vernberg and Costlow, 1966; Crane, 1975). Studies on other crab species, such as *Uca forcipata* and *U. lactea* populations in Thailand, have shown similar ratios of left- and right-handedness (Frith and Brunenmeister, 1983). Furthermore, it has been suggested that Southeast Asian populations tend to exhibit brachychelous morphism, while American populations are dominated by leptochelous morphism (Frith and Frith, 1977b; Crane, 1975). The occurrence of leptochelous, brachychelous, and symmetric chelae in *C. carnifex* may be influenced by genetic factors associated with the development of this structure or by the influence of sex-related genes.

5 Conclusion

Morphologically and morphometrically, *C. carnifex* exhibits sexual dimorphism, with notable differences in various body structures between males and females. One significant phenomenon is the difference in abdomen first structure width (ASW) between males and females. Additionally, the correlation coefficient (r^2) highlights a stronger association between body weight (BW) and carapace width (CW) compared to BW and carapace length (CL), indicating that variations in body weight are predominantly influenced by changes in carapace width. In conclusion, *C. carnifex* demonstrates a sex-related relative growth in different body parts, with females exhibiting the largest body weight and males showing the smallest body weight. The observed sex ratio of 0.9:1 suggests that females outnumber males in the population. The occurrence of leptochelous, brachychelous, and symmetric chelae in *C. carnifex* may be influenced by genetic factors associated with the development of these structures or by the influence of sex-related genes. Further genetic analyses are warranted in *C. carnifex* populations as a complementary approach to identify possible phenotypic correlations with genetic differentiation between the Parangipettai population and nearby populations. This would provide valuable insights into the underlying mechanisms driving morphological variations and sexual dimorphism in this species.

Acknowledgements

The authors wish to thank the support of the Ministry of Environment, Forests and Climate Change, New Delhi (F.No.22018-30/2019-CS (Tax)) for funding.

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